Targ Oncol (2016) 11:317–327
DOI 10.1007/s11523-015-0394-5

ORIGINAL RESEARCH ARTICLE

Phase 1 Study of Monotherapy with KHK2866, an Anti-Heparin-Binding Epidermal Growth Factor-Like Growth Factor Monoclonal Antibody, in Patients with Advanced Cancer

John Sarantopoulos[1] · Monica M. Mita[2] · Michael J. Birrer[3] · Lee D. Cranmer[4] · Luis T. Campos[5] · Xiaoping Zhang[6] · Penelope Bristow[6] · Hidekuni Kaito[6] · Vincent Strout[6] · Luis H. Camacho[5]

Published online: 27 October 2015

Abstract
Background KHK2866 is a recombinant, humanized, non-fucosylated, monoclonal antibody directed at heparin-binding epidermal growth factor-like growth factor (HB-EGF).
Objective To determine the safety, tolerability, maximum tolerated dose (MTD), pharmacokinetics, pharmacodynamics, potential immunogenicity, and preliminary clinical efficacy of KHK2866 monotherapy in patients with advanced and refractory cancer in a first-in-human, phase 1 study.
Materials and Methods Using a standard 3+3 dose-escalation design, 20 patients received KHK2866 (0.3, 1, and 3 mg/kg) intravenously once weekly. Two additional patients received 0.1 mg/kg in a cohort which was subsequently added following protocol amendment.
Results The first three patients enrolled experienced grade 2 hypersensitivity (acute infusion reactions) after the first dose of KHK2866. After prophylactic treatment with an H1-blocker and corticosteroids in subsequently recruited patients, two grade 2 hypersensitivity reactions were observed in the remaining 19 patients. Grade 2/3 neurotoxicity appeared to be dose-limiting at 3 mg/kg in the original dose-escalation cohorts (n=2), at 1 mg/kg in the MTD dose expansion cohort (n=1), and at 0.1 mg/kg (n=1). Neurotoxicity was manifested as complex partial seizure activity, aphasia, and confusion

Electronic supplementary material The online version of this article (doi:10.1007/s11523-015-0394-5) contains supplementary material, which is available to authorized users.

✉ John Sarantopoulos
sarantopoulo@uthscsa.edu

Monica M. Mita
Monica.Mita@cshs.org

Michael J. Birrer
mbirrer@partners.org

Lee D. Cranmer
Lcranmer@uacc.arizona.edu

Luis T. Campos
Lcampos@oncologyconsultants.com

Xiaoping Zhang
azhang@kyowa-kirin-pharma.com

Penelope Bristow
penny@coreclinicalsolutions.com

Hidekuni Kaito
hidekuni.kaito@fujifilmkyowakirin-biologics.com

Vincent Strout
vstrout@kyowa-kirin-pharma.com

Luis H. Camacho
lhcamacho@mac.com

[1] Institute for Drug Development, Cancer Therapy and Research Center at University of Texas Health Science Center San Antonio, 4th Floor, Zeller Building, 7979 Wurzbach Road, San Antonio, TX 78229, USA

[2] Samuel Oschin Comprehensive Cancer Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA

[3] Gillette Center of Gynecologic Oncology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

[4] University of Arizona Cancer Center, Tuscon, AZ, USA

[5] Oncology Consultants, Houston, TX, USA

[6] Kyowa Hakko Kirin Pharma, Inc., Princeton, NJ, USA

after first-dose administration. Pharmacokinetic exposure to KHK2866 increased proportionally to dose. Mean elimination half-life was 71.9–118 h over the dose range from 0.3 to 3 mg/kg. All KHK2866 doses decreased serum free HB-EGF levels, generally below the lower limit of quantification. *Conclusions* The study was terminated because of neuropsychiatric toxicity. The only predictive factor for neuropsychiatric toxicity was administration of KHK2866. These effects were reversible, but were not predictable. Their etiology is not presently understood. [Study registered at ClinicalTrials.gov #NCT0179291]

Key Points

KHK2866, an anti-heparin-binding epidermal growth factor-like growth factor monoclonal antibody, was evaluated in a first-in-human phase 1 study in patients with advanced cancer.

The study and development of KHK2866 was abandoned because of the dose-limiting neuropsychiatric toxicity.

KHK2866 decreased soluble HB-EGF levels in a non-dose dependent manner in most patients.

1 Introduction

The epidermal growth factor (EGF) family of receptors (ErbB1, ErbB2, ErbB3, and ErbB4) is widely recognized to play a key role in many human malignancies [1, 2]. Blockade of the EGF receptor (EGFR/ErbB1) and human epidermal growth factor receptor 2 (ErbB2/HER2) with therapeutic monoclonal antibodies (mAbs) (e.g. cetuximab, panitumumab, trastuzumab) and small molecule tyrosine kinase inhibitors (e.g. erlotinib, gefitinib, lapatinib) has proven effective in the treatment of various cancers [3–8]. These agents are presumed to act, at least in part, by blocking the signaling of various ligands via their cognate ErbB receptors, thereby inhibiting downstream signaling that promotes cell proliferation, invasion, survival, and angiogenesis. Less attention has been paid to the potential for therapeutic targeting of the family of EGFR ligands, although evidence supports this concept [9, 10]. Furthermore, the concept of ligand targeting has proven therapeutically successful in a variety of cancers with bevacizumab, an mAb that binds vascular endothelial growth factor A [11–14].

Among the various ErbB ligands, heparin-binding epidermal growth factor-like growth factor (HB-EGF) binds to EGFR and ErbB4 [15]. HB-EGF is synthesized as a transmembrane protein (pro-HB-EGF); this is cleaved by metalloproteases to release the soluble, mitogenically active form of HB-EGF, a process termed ectodomain shedding [16]. HB-EGF promotes oncogenic transformation, cellular proliferation, motility, migration and invasion, and metastasis (for review see Miyamoto et al. [17]). HB-EGF overexpression has been implicated in tumor progression in a range of cancers, such as pancreatic, hepatic, colon, gastric, esophageal, bladder, melanoma, breast, and ovarian [10, 18–25]. Furthermore, ovarian cancer patients with high HB-EGF expression have a less favorable prognosis than those with low expression, indicating that HB-EGF may play a pivotal role in the progression of ovarian cancer [26]. HB-EGF has also been implicated in resistance to chemotherapeutic agents (e.g. paclitaxel, cisplatin, 5-fluorouracil, doxorubicin) in vitro using various cancer cell lines and in vivo in xenograft models [27–30]. HB-EGF inactivation, therefore, presents a potential strategy for cancer therapy.

KHK2866 is a recombinant, humanized, non-fucosylated immunoglobulin G1 (IgG1)κ mAb directed at HB-EGF. Defucosylation of the carbohydrate residues on the gamma heavy chain results in 10- to 100-fold enhancement of antibody-dependent cellular cytotoxicity activity (ADCC) [31]. KHK2866 is derived from a parental murine mAb KM3566 [32]. KM3566 shows high binding affinity for membrane-bound pro-HB-EGF and neutralizing activity against soluble HB-EGF; it exhibits potent in vivo anti-tumor activity in murine models inoculated with HB-EGF-expressing ovarian cancer cells via direct inactivation of soluble HB-EGF and also immunotherapeutically via ADCC [32, 33]. KHK2866 has demonstrated a high degree of cytotoxicity against mucinous cystadenocarcinoma and ES-2 ovarian cancer cell lines and significant anti-tumor activity either as a single agent or additively in combination with paclitaxel, gemcitabine, carboplatin, or doxorubicin in an HB-EGF-positive human ovarian cancer xenograft model [data on file, Kyowa Hakko Kirin Pharma, Inc., Princeton, NJ].

The aim of the current first-in-human phase 1a study was to determine the safety, tolerability, maximum tolerated dose (MTD), pharmacokinetics, pharmacodynamics, potential immunogenicity, and preliminary clinical efficacy of KHK2866 administered by intravenous (IV) infusion as monotherapy in patients with advanced cancer. A phase 1b study had been planned to determine the MTD of KHK2866 in combination with selected chemotherapies in patients with advanced epithelial ovarian cancer. Since the study was terminated during the phase 1a component due to unacceptable neuropsychiatric toxicity, the phase 1b study was not undertaken.

2 Materials and Methods

The study was conducted in accordance with the Declaration of Helsinki and International Conference for Harmonization of Good Clinical Practice guidelines and registered at

ClinicalTrials.gov (NCT0179291). All patients provided written informed consent prior to study registration. The protocol and its subsequent amendments were approved by the institutional review board at each of the six participating study centers (Massachusetts General Hospital, MA, USA; Oncology Consultants, Houston, TX, USA; University of Arizona Cancer Center, Tucson, AZ, USA; Institute for Drug Development, Cancer Therapy and Research Center at University of Texas Health Science Center, San Antonio, TX, USA; USC Norris Comprehensive Cancer Center, Los Angeles, CA, USA; and Samuel Oschin Comprehensive Cancer Institute, Los Angeles, CA, USA).

2.1 Study Design

The primary objective was to determine the safety, tolerability, and MTD of KHK2866. Secondary objectives were to characterize the pharmacokinetics of KHK2866, to determine the pharmacodynamic profile for KHK2866, to screen for potential development of antibodies against KHK2866, and to describe preliminary anti-tumor activity.

As this was the first-in-human study of KHK2866, the starting dose level was based on a toxicology study in cynomolgus monkeys (data on file, Kyowa Hakko Kirin Pharma, Inc.), which showed the highest non-severely toxic dose (HNSTD) and no observed adverse effect level (NOAEL) was ≥100 mg/kg (1200 mg/m^2) IV twice weekly. This yielded a starting dose for humans of 200 mg/m^2 (5.4 mg/kg) using the standard safety factor of one-sixth the HNSTD. The selected starting dose of 0.3 mg/kg weekly provided a 216-fold safety margin when compared to the HNSTD and NOAEL, taking into account the more frequent dosing regimen in the toxicology study.

This study was conducted using a standard 3+3, open-label, dose-escalation design. Patients with advanced solid tumors, including females with epithelial ovarian cancer (EOC), received KHK2866 monotherapy. The initial plan was to administer increasing doses of KHK2866 (0.3, 1, 3, and 10 mg/kg weekly) for 4 weeks (cycle 1). Dose-limiting toxicity (DLT) was determined over cycle 1. Patients discontinuing because of adverse events (AEs) or disease progression, or not receiving all scheduled doses of KHK2866 during cycle 1 were replaced by enrollment of new patients in the relevant cohort.

KHK2866 (Kyowa Hakko Kirin Pharma, Inc.) was administered by IV infusion in 50 or 150 mL of 0.9 % saline over 60 minutes using an infusion pump with a low-protein-binding, 0.22-μm, in-line filter. The volume of saline used was selected to ensure an infused drug concentration ranging from >0.1 to <8 mg/mL. There was no requirement for fasting prior to or after administration. Patients were continuously treated with KHK2866 once weekly and a treatment period of 4 weeks was defined as one cycle. Patients were allowed to continue treatment for up to six cycles or until the disease progression, decline in Eastern Cooperative Oncology Group (ECOG) performance status score ≥2, development of unacceptable toxicity, grade 3/4 infusion reaction, any potentially life-threatening, therapy-related event regardless of grade, protocol non-adherence, or withdrawal of consent. Patients could continue treatment beyond six cycles if they experienced a best response of at least stable disease.

DLT was defined as the occurrence of any of the following toxicities considered as possibly, probably, or definitely related to KHK2866: grade 4 anemia or thrombocytopenia; grade 4 neutropenia for ≥5 days; grade 3/4 neutropenia with fever (≥38.5 °C) for ≥4 h; grade ≥3 non-hematologic toxicity (except for grade 3 nausea/vomiting or diarrhea reduced to grade ≤2 within 24 h with medical management, or grade 3 laboratory AE that is asymptomatic and rapidly reversible [returning to baseline or grade ≤1 within 7 days or prior to next administration of KHK2866]); and any other toxicity leading to treatment interruption for ≥2 weeks or representing a clinically significant hazard in the view of the investigator.

Once the MTD was determined, an expansion cohort was planned, recruiting patients with platinum-resistant EOC or locally unresectable and/or metastatic cancer of squamous histology. Only one patient was eventually included in the MTD expansion cohort (see "Results" and "Discussion").

An additional fourth cohort of 0.1 mg/kg was subsequently added, which was less than the original 0.3 mg/kg starting dose of the study. This was to allow reassessment of the MTD (see "Results" and "Discussion"). Administered as monotherapy, a dose of 0.1 mg/kg exhibited similar, significant anti-tumor activity to 1.0 mg/kg in an ovarian carcinoma xenograft SCID mouse model (T/C minimum values of 0.56 and 0.54, respectively) (data on file, Kyowa Hakko Kirin, Inc.). The use of 0.1 mg/kg in the de-escalation cohort was therefore considered justified on a pharmacological basis.

2.2 Patients

For the dose-escalation cohorts, eligible patients included adults (≥18 years) with histologically or cytologically documented, measurable or non-measurable, advanced primary or recurrent solid cancer unresponsive to standard therapy or for which no standard therapy was available. Patients with advanced, recurrent, platinum-resistant EOC may have received no more than two prior cytotoxic chemotherapy regimens. Chemotherapy regimens consisting of different platinum agents and/or different taxanes were considered a single platinum/taxane regimen.

For the MTD expansion cohort, eligible patients were restricted to those with advanced, recurrent, platinum-resistant EOC (as described above) or locally unresectable and/or metastatic squamous solid cancer. All patients had to have an ECOG score ≤2 at entry, a life expectancy of at least 3 months, and preserved organ function. Full inclusion/exclusion criteria are provided as supplementary data (available online).

2.3 Safety and Clinical Assessment

Demographic and medical/cancer histories were recorded at screening. Physical examination was undertaken during screening, on days 1, 8, and 15 of cycle 1, on day 1 of subsequent cycles, at the end of treatment, and at 30-day follow-up. Complete blood count with differential and clinical chemistry were determined during screening, on days 1, 8, 15, and 22 of cycles 1 and 2, on days 1 and 15 of subsequent cycles, at the end of treatment, and at 30-day follow-up. Urinalysis, blood coagulation factors, ECOG score, weight, and serum CA-125 (for patients with ovarian carcinoma) were measured at screening, on day 1 of each treatment cycle, at the end of treatment, and at 30-day follow-up. Vital signs were recorded at all visits. Pregnancy testing was performed during screening. Diffusion capacity, pulse oximetry, chest computed tomography scan, and brain magnetic resonance imaging were determined during screening and, if appropriate, for subsequent response assessment. ECG was undertaken during screening, pre-dose and 1 hour after the conclusion of the infusion on days 1 and 22, and at the end of treatment. Multigated acquisition scan or echocardiogram were obtained during screening, at the end of cycle 1, at the end of every two cycles thereafter, and at the end of study participation.

AEs were recorded following observation by the investigator during clinic visits or in response to non-leading questioning, spontaneous reporting by the patient, or on the basis of clinical or laboratory tests. They were graded by National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) v4.0. Treatment-related AEs were those classified as possibly, probably, or definitely related to KHK2886. The safety analysis population included all patients who received at least one dose of KHK2886. Serious AEs (SAEs) were reported in an expedited manner.

Blood samples for assessment of anti-KHK2866 antibodies were collected prior to infusion of KHK2866 on days 1 and 15 of cycle 1, on day 1 of cycle 2, on day 1 of every subsequent two cycles, at the end of treatment, and at 30-day follow-up. Pre- and post-treatment serum samples were assayed for anti-KHK2866 antibodies with a validated electrochemiluminescent (ECL) assay. A patient was scored as having a positive anti-KHK2866 antibody if any sample was found to have a positive screening assay which was then confirmed by an immunodepletive confirmation assay.

2.4 Response Assessment

Best overall response was determined in the efficacy evaluable population. This was defined as those patients with baseline and at least one on-study tumor assessment. Responses in those with solid tumors other than EOC were assessed according to Response Evaluation Criteria in Solid Tumors (RECIST guidelines version 1.1) [34]. In patients with EOC, responses were assessed by the combination of RECIST and/or serum CA-125 criteria (Gynecologic Cancer Intergroup [35]).

Patients with measurable disease were evaluated by imaging and physical examination at 8 weeks after the first dose of KHK2866. This was repeated every 8 weeks. Confirmation of response was required not less than 4 weeks following initial response documentation. In patients with EOC, serum CA-125 response was determined every 4 weeks.

Progression-free survival (PFS) and response duration were estimated using the Kaplan-Meier method. PFS was defined as the time from the date of the first dose of study drug until the first date on which progressive disease or death due to any cause was documented; patients were censored at the date of the last evaluable tumor assessment for patients who were lost to follow-up or who were alive at the time of analysis.

2.5 Pharmacokinetics

Blood samples were taken pre-dose, at the end of infusion, and at 1, 3, 6, 24, 48, 72, 96, and 168 h after the end of infusion of KHK2866 after dosing on days 1 and 22 of cycle 1. Serum samples were analyzed for KHK2866 using a validated sandwich ECL method, which employed an anti-KHK2866 murine mAb as both the capture antibody and a ruthenium-labeled detection antibody. The standard curve ranged from 5 to 2000 ng/mL with a lower limit of quantification (LLOQ) of 10 ng/mL. The assay accuracy (relative error) and precision (coefficient of variation for the mean) were within acceptance criteria (< ± 20 %) for all quality control samples (98.7 %) except for two samples (1.3 %) which showed a relative error of 28.0 % and 29.0 %, respectively. Pharmacokinetic parameters including area under the serum concentration-time curve from time zero to 7 days after dosing ($AUC_{0–7days}$), maximum (C_{max}) and trough (C_{trough}) serum concentration, time to C_{max} (T_{max}), and elimination half-life ($t_{1/2}$) were calculated using non-compartmental methods with Phoenix® WinNonlin® software (Version 6.3, Pharsight, A Certara™ Company, Mountain View, CA, USA).

2.6 Pharmacodynamics

Blood samples were taken pre-dose, and at 3, 24, and 72 h after the end of KHK2866 infusion on days 1 and 22. Pre-dose samples were also taken on days 8 and 15. Serum samples were analyzed for free serum HB-EGF by an immuno-PCR method that employed an anti-HB-EGF mAb for capture and DNA-conjugated anti-HB-EGF goat polyclonal antibody for detection. The standard curve ranged from 10 to 200 ng/mL with an LLOQ of 20 ng/mL [36].

2.7 Statistics

Safety, efficacy, pharmacokinetic, and pharmacodynamic data were summarized by descriptive statistics. Power analysis, in which the natural logarithm (Ln) of C_{max} and $AUC_{0–7days}$ values were regressed against Ln dose values, was conducted to assess dose proportionality of KHK2866. The 95 % confidence interval (CI) was constructed for the β value and inclusion of a null value of 1.0 within the 95 % CI was considered to suggest that a dose-proportional increase could not be ruled out.

3 Results

3.1 Patient Characteristics

The study is complete and was conducted between 1 February 2011 and 21 November 2012. The baseline clinical and demographic characteristics of 22 patients (17 non-EOC, 5 EOC) are summarized in Table 1. Patient disposition and drug exposure are summarized in Table 2. All 22 patients were included in the safety population and 18 were evaluable for efficacy. The most common reason for discontinuation from the study other than progressive disease (n=9, 40.9 %) was unacceptable AE/toxicity (n=9, 40.9 %).

Table 1 Baseline clinical and demographic characteristics

Characteristic	Total (N=22)
Median age, years (min, max)	60.6 (32, 76)
Gender, *n* (%)	
Male	13 (59.1)
Female	9 (40.9)
Race, *n* (%)	
White	20 (90.9)
Asian	2 (9.1)
ECOG performance status, *n* (%)	
0	9 (40.9)
1	11 (50.0)
2	2 (9.1)
Epithelial ovarian cancer, *n* (%)	5 (22.7)
Non-epithelial ovarian cancer, *n* (%)	17 (77.3)
Primary tumor site of non-epithelial ovarian cancers, *n* (%)	
Colorectal	8 (36.4)
Liver	2 (9.1)
Other[a]	7 (31.8)
Median disease duration, months (min, max)	32.30 (3.0, 144.0)
Prior cancer therapy, *n* (%)	
Chemotherapy	21 (95.5)
Surgery	21 (95.5)
Radiotherapy	8 (36.4)
Median no. of prior chemotherapies (min, max)	5.0 (1, 9)

ECOG, Eastern Cooperative Oncology Group

[a] Includes squamous cancers of the ampulla of vater, esophagus, eyelid, lung, pancreas, skin, and uterus (each n=1)

3.2 Dose-Limiting Toxicity

No DLTs were identified in the initial enrollment in cohorts 1 and 2 (0.3 and 1 mg/kg weekly, respectively). One patient in cohort 1 had grade 2 metabolic encephalopathy 4 days after the first infusion of KHK2866 that was attributed to hypothyroidism and considered unlikely to be drug-related: it was, therefore, not assessed as a DLT. The first DLTs were observed in cohort 3 (3 mg/kg weekly), therefore, exceeding the MTD as two of six patients experienced serious, reversible neurological events (grade 3 confusional state with grade 3 aphasia in one patient and grade 3 seizure in another). Following these observations, additional patients were recruited to cohort 2 (1 mg/kg weekly) according to the 3+3 design: one patient experienced a DLT (grade 3 tumor lysis syndrome). KHK2866 1 mg/kg weekly was provisionally established as the MTD and became the accepted dose for the MTD expansion cohort. One patient was recruited to the MTD expansion cohort who then experienced DLT (grade 3 depressed level of consciousness, grade 3 fever, and grade 2 seizure). At this point, enrollment was halted by the sponsor to evaluate the observed neuropsychiatric AEs; the protocol was revised prior to additional enrollment (see "Discussion").

Because of these toxicities, a new dose level was established (0.1 mg/kg weekly), which was less than the original 0.3 mg/kg weekly starting dose of the study and was 10-fold less than the original MTD (1 mg/kg weekly) to allow the reassessment of the MTD and gain additional information on pharmacokinetic/pharmacodynamic parameters. This cohort was expanded, with stopping rules for safety added, to a maximum of 30 patients. Two patients were enrolled at the 0.1 mg/kg weekly dose level, one of whom experienced neurological DLT (grade 2 ataxia plus grade 3 extrapyramidal disorder). After internal review of the KHK2866 safety profile across all doses, the study was terminated.

3.3 Safety

The first three patients enrolled into the study in cohort 1 (0.3 mg/kg weekly) experienced grade 2 hypersensitivity (acute infusion reactions) from 7 min to 2 h after completion of KHK2866 infusion, which responded to administration of diphenhydramine and corticosteroid. These reactions were characterized by various combinations of fever, chills, rigors, sinus tachycardia, arterial oxygen desaturation, and/or hypertension. Following protocol amendment, all subsequently

Table 2 Patient disposition and drug exposure

	KHK2866 cohort					
	Cohort 0 0.1 mg/kg (*n*=2)	Cohort 1 0.3 mg/kg (*n*=5)	Cohort 2 1 mg/kg (*n*=8)[a]	Cohort 3 3 mg/kg (*n*=6)	Expansion cohort 1 mg/kg (*n*=1)	Total (*N*=22)
Patient disposition, *n* (%)						
Safety population	2	5	8	6	1	22 (100.0)
Efficacy population	1	4	6	6	1	18 (81.8)
Reason for withdrawal						
Unacceptable AE/toxicity	1	2	3	2	1	9 (40.9)
Disease progression	0	2	4	3	0	9 (40.9)
Consent withdrawal	1	0	0	1	0	2 (9.1)
Decrease in ECOG score ≥2	0	1	1	0	0	2 (9.1)
Drug exposure, mean±SD						
Cycles initiated, *n*	1.0±0.00	2.2±2.17	3.9±4.05	1.7±0.82	1.0	2.5±2.79
Total KHK2866 doses administered, *n*	1.0±0.00	7.6±9.61	13.1±14.71	5.2±4.79	1.0	8.0±10.70
Actual KHK2866 dose, mg	4.79±0.09	23.07±7.71	74.93±35.43	220.78±51.14	67.00	96.18±88.14
Percent dose intensity[b]	75.0±35.36	100.0	90.7±13.22	90.4±20.02	100.0	91.7±16.14

AE, adverse events; ECOG, Eastern Cooperative Oncology Group; SD, standard deviation

[a] One patient received the first two doses of cycle 1 at 0.3 mg/kg instead of the assigned dose of 1 mg/kg

[b] Calculated as actual dose/planned dose×100 %

recruited patients received prophylactic premedication regardless of KHK2866 dose. Premedication consisted of administration of an H1-blocker (e.g. diphenhydramine 25–50 mg) plus an IV corticosteroid (e.g. dexamethasone 10 mg) 30 min prior to each of the four scheduled KHK2866 infusions in cycle 1. Diphenhydramine dosing was 50 mg IV for patients ≥50 kg and <75 years, or 25 mg IV for those <50 kg or ≥75 years. Acetaminophen (650–1000 mg 30 min prior to infusion) could be added to the premedication regimen at the discretion of the investigator. Beginning with the cycle 2 day 1 infusion, the investigator could start to taper the steroid and eventually stop the steroid altogether, if tolerated. It was recommended that the H1-blocker continue to be administered, although the investigator could also taper the H1-blocker if it was deemed to be producing toxicity. Among the additional 19 patients who received this premedication regimen following protocol amendment, two experienced grade 2 hypersensitivity reactions following the first dose of KHK2866. These reactions tended to be milder than non-premedicated hypersensitivity reactions and promptly responded to additional H1-blocker, corticosteroid, and/or acetaminophen. There were no hypersensitivity reactions associated with second or subsequent infusions of KHK2866. Because one patient had a delay as long as 6 h between the end of the first infusion and the onset of a hypersensitivity reaction, it was subsequently mandated that all patients be observed for no less than 6 hours following the first infusion and at least 1 h following the completion of subsequent KHK2866 infusions. There were no grade 3/4 hypersensitivity reactions in any patients.

A summary of treatment-emergent AEs is provided in Table 3. A total of 30 SAEs were reported in 14 patients (63.6 %): six of these patients had treatment-related SAEs, which included all five patients with DLTs plus an additional patient with grade 2 pyrexia. Among the SAEs, there were four patient deaths during the study, none of which were considered related to treatment (sepsis, cardiopulmonary failure, respiratory failure plus sepsis, and CNS metastases).

The most notable AEs were the severe neurological and psychiatric events which were determined to be DLTs for KHK2866. These four events occurred after a single dose and resolved within 10 days. A search of all AEs revealed a total of 16 patients (72.7 %) with at least one neurological or psychiatric AE: most of these events (32 of 39 events, 82.1 %) were grade 1/2 and seven events (17.9 %) were grade 3. None of the patients developed an anti-KHK2866 antibody response during the study, although one patient was positive during screening.

3.4 Anti-tumor Activity

Best overall response was stable disease in nine patients (50.0 %) among the 18 evaluable patients, three of which were confirmed (16.7 %). The three cases with confirmed stable disease occurred patients with non-EOC of squamous origin

Table 3 Treatment-emergent adverse events

	No. of patients (%)					
	KHK2866 cohort					
	Cohort 0 0.1 mg/kg weekly (n=2)	Cohort 1 0.3 mg/kg weekly (n=5)	Cohort 2 1 mg/kg weekly (n=8)[a]	Cohort 3 3 mg/kg weekly (n=6)	Expansion cohort 1 mg/kg weekly (n=1)	Total (N=22)
AE	2	5	8	5	1	21 (95.5)
Treatment-related AE[b]	2	4	6	5	1	18 (81.8)
AE grade ≥3	1	3	6	4	1	15 (68.2)
Treatment-related AE grade ≥3[b]	1	0	1	2	1	5 (22.7)
Serious AE	2	3	4	4	1	14 (63.6)
Treatment-related serious AE[b]	2	0	1	2	1	6 (27.3)
AE leading to discontinuation of KHK2866	1	1	4	3	1	10 (45.5)
Death[c]	0	1	2	1	0	4 (18.2)
AE occurring in ≥2 patients overall by preferred term[d]						
Hypersensitivity	0	3	2	0	0	5 (22.7)
Pyrexia	1	1	1	0	1	4 (18.2)
Dyspnea	0	0	1	1	1	3 (13.6)
Seizure	0	0	0	1	1	2 (9.1)
Aphasia	1	0	0	1		2 (9.1)
Dizziness	0	0	0	1	1	2 (9.1)
Nausea	0	0	1	1	0	2 (9.1)
Stomatitis	0	1	1	0	0	2 (9.1)
Musculoskeletal pain	1	0	1	0	0	2 (9.1)
AE grade ≥3 occurring in ≥2 patients overall by preferred term[d]						
Fatigue	0	1	1	0	0	2 (9.1)
Sepsis	0	1	1	0	0	2 (9.1)
INR increased	0	1	1	0	0	2 (9.1)
Pleural effusion	0	1	0	1	0	2 (9.1)

AE, adverse events; INR, International normalized ratio

[a] One patient received the first two doses of cycle 1 at 0.3 mg/kg instead of the assigned dose of 1 mg/kg

[b] Considered by the investigator as possibly, probably, or definitely related to treatment

[c] All deaths were unrelated to study medication

[d] Coded by MedDRA version 13.1

(pulmonary in 2 and cutaneous in 1). The duration of stable disease ranged from 15 to 88 days in the six patients with an unconfirmed response and was 163, 178, and 355 days, respectively, in the three patients with a confirmed response. Median PFS was 1.93 months (95 % confidence interval, 1.70–2.97 months).

3.5 Pharmacokinetics

Mean serum KHK2866 concentrations at different doses (0.1, 0.3, 1, and 3 mg/kg) during cycle 1 are illustrated in Fig. 1, and mean pharmacokinetic parameters are summarized in Table 4. Mean T_{max} ranged from 1.38 to 4.03 h. Serum KHK2866 concentrations ($AUC_{0-7days}$) showed 1.3- to 2.5-fold accumulation following repeated once-weekly administration for 4 weeks. Exposure to KHK2866 based on C_{max} and $AUC_{0-7days}$ values increased in a dose proportional manner over the dose range from 0.1 to 3 mg/kg. Power analysis in which the Ln C_{max} or Ln $AUC_{0-7days}$ values were regressed against Ln dose across all patients yielded β values of 1.01 (95 % CI: 0.861–1.16) and 1.09 (95 % CI: 0.880–1.29) for C_{max}, and 1.11 (95 % CI: 0.921–1.29) and 1.42 (95 % CI: 0.884–1.97) for $AUC_{0-7days}$ after the first and fourth dose, respectively. Mean $t_{1/2}$ ranged from 71.9 to 118 h and appeared independent of dose and repeated dosing. At the highest dose studied (3 mg/kg), C_{max}, C_{trough}, and $AUC_{0-7days}$ were 124± 26.8 μg/mL, 45.4±16.5 μg/mL, and 11,900±3920 μg•h/mL, respectively, after the fourth dose in cycle 1.

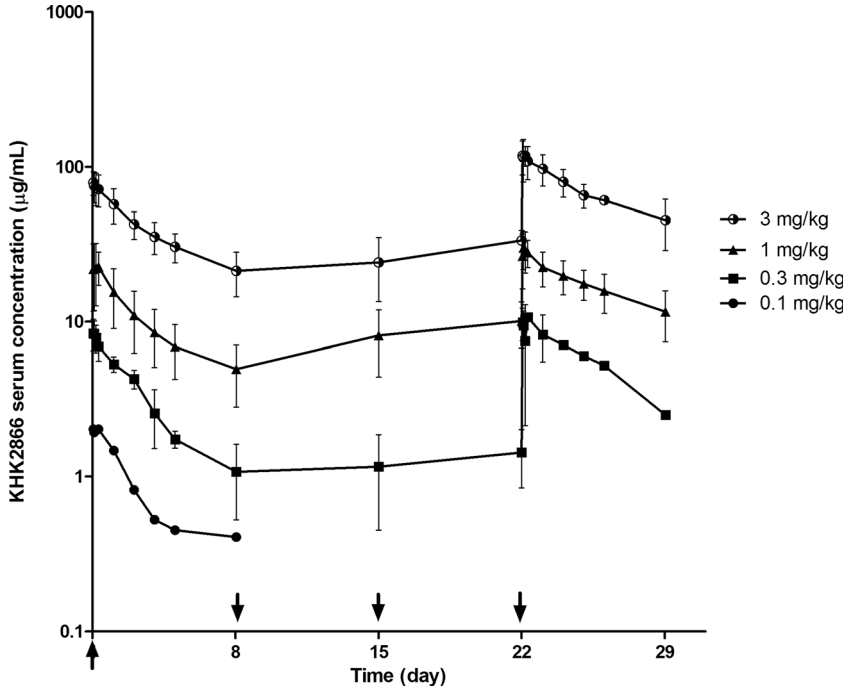

Fig. 1 Mean serum KHK2866 concentration-time profiles during cycle 1. Arrows indicate time of weekly dose administration. Error bars indicate standard deviation of the mean. Note: post-dose determination of serum KHK2866 was not performed following the second and third dose except for trough values immediately prior to dose administration indicated at day 14 and 21 time points

3.6 Pharmacodynamics

Individual serum HB-EGF concentrations following the first dose of KHK2866 during week 1 of cycle 1 are detailed as supplementary data (available online). The majority of patients (18 of 22; 82 %) had measurable pre-dose HB-EGF levels above the LLOQ. This was rapidly reduced at 3 h post-dose, when 18 of 22 patients (82 %) had undetectable HB-EGF levels, and generally remained undetectable in these patients until the end of the dose interval (1 week). Fifteen of the 18 patients with measurable pre-dose HB-EGF levels had an undetectable level on at least one post-dose time point

Table 4 Pharmacokinetics of KHK2866

KHK2866 dose	Week	No. of patients	Mean±SD					
			T_{max} (h)	C_{max} (μg/mL)	C_{trough} (μg/mL)	$AUC_{0–7days}$ (μg•h/mL)	$t_{1/2}$ (h)	R
0.1 mg/kg[a]	1	2	4.03	2.12	0.407[b]	117	75.2	–
0.3 mg/kg	1	5	2.44±2.61	8.73±1.58	1.07±0.54[c]	441±128	71.9±47.3[g]	–
	4	3	1.38±0.543	10.1±2.35	2.50[d]	694±545	72.5[d]	1.28±0.94
1 mg/kg[h]	1	9	2.67±1.98	23.9±9.63	4.94±2.13[e]	1,470±519	118±45.4	–
	4	6	2.59±1.60	32.4±7.98	11.6±4.18[f]	2,700±599	118±17.4[c]	2.51±1.54
3 mg/kg	1	6	2.41±2.30	80.5±14.4	21.3±6.84[c]	5,330±1,500	112±39.2	–
	4	3	3.01±1.01	124±26.8	45.4±16.5[c]	11,900±3,920	102[d]	1.94±0.19

$AUC_{0–7days}$, area under the serum concentration-time curve from time zero to 7 days after dosing; C_{max}, maximum serum concentration; C_{trough}, trough serum concentration; R, accumulation ratio (fourth dose/first dose for $AUC_{0–7days}$); SD, standard deviation; T_{max}, time to C_{max}; $t_{1/2}$, elimination half-life

[a] Week 4 data for 0.1 mg/kg was not available

[b] n=1

[c] n=3

[d] n=2

[e] n=6

[f] n=5

[g] n=4

[h] One patient received the first two doses of cycle 1 at 0.3 mg/kg instead of the nominal dose of 1 mg/kg. The pharmacokinetic data for this patient are reported at the nominal dose without correcting for the dosing error

during the dose interval (and usually throughout the dose interval): three of the neuropsychiatric DLTs occurred among these 15 patients and the remaining neuropyschiatric DLT occurred in a patient whose post-dose HB-EGF was not greatly reduced at any time point.

4 Discussion

The MTD for KHK2866 could not be established due to the number and type of AEs observed across dose levels. Two major safety issues were identified in the clinical study: acute infusion reactions and dose-limiting central neuropsychiatric AEs.

Hypersensitivity (acute infusion reactions) was mitigated by implementing a prophylactic premedication regimen for all patients after all of the first three patients treated experienced grade 2 hypersensitivity after their first drug dose. Among the 19 additional patients who received the specified premedication regimen beginning with their first infusion, an additional two patients experienced grade 2 hypersensitivity, which tended to be milder than non-premedicated reactions and promptly responded to additional H1-blocker, corticosteroid, and/or acetaminophen. There were then no reactions associated with second or subsequent infusions.

Neurotoxicity appeared as a DLT at 3 mg/kg in the original dose-escalation cohorts, at 1 mg/kg in the MTD dose expansion cohort, and at 0.1 mg/kg in a decreased dose cohort which was subsequently added following protocol amendment. The neurotoxicity was typically manifested as complex partial seizure activity, aphasia, and confusion after first-dose administration and was reversible following discontinuation of KHK2866. No pattern of patient gender, age, pre-existing condition, or pharmacokinetic or pharmacodynamic response could be identified that predicted the likelihood of neuropsychiatric AEs other than the first exposure to KHK2866. No dose relationship was observed and a risk-mitigation strategy was not readily apparent. Patients with a known history of brain metastases and primary brain neoplasm and those with leptomeningeal disease had already been excluded from the study in the original exclusion criteria. Despite the implementation of more restrictive exclusion criteria, including the requirements for specific elements during the screening neurological examination, similar neurotoxicity was observed even at the reduced dose of 0.1 mg/kg.

An analysis of the pharmacodynamic response of free serum HB-EGF following infusion of KHK2866 revealed that most patients, across all dose levels, experienced a prompt (within 3 h from the start of infusion) and sustained drop in their free serum HB-EGF level to below the LLOQ (<20 pg/mL). Fifteen of the 22 patients had measurable HB-EGF at the pre-dose time point (day 1) that dropped below LLOQ at some time point after dosing. Three of those 15 patients had neuropsychiatric DLTs and 12 patients did not, while the remaining neuropsychiatric DLT was observed in a patient whose post-dose HB-EGF was not greatly reduced. Therefore, it appears that the neuropsychiatric DLTs may not be correlated with soluble HB-EGF reduction but these numbers are too small to draw any conclusion on the correlation between the level of free serum HB-EGF and development of these AEs.

HB-EGF is widely expressed in both the developing and adult rodent CNS, including neurons and neuroglia in the hippocampus, cerebellum, cerebral cortex, subventricular area, and brain stem nuclei [37, 38]. In an experimental mouse model of lysolecithin-induced demyelination in the corpus callosum, intranasal HB-EGF administration has been shown to induce proliferation of neural progenitor cells in the subventricular zone, as well their migration towards demyelinated lesions in the corpus callosum [39]. Moreover, neurobehavioral testing in a conditional HB-EGF knockout mouse model (in which the knockout is limited to the ventral forebrain and allows survival into the adult/postnatal period) revealed abnormal psychomotor behaviors, less social interaction, and impairment of spatial memory and fear learning versus control mice [40, 41]. These studies also reported lower dendritic spine density and differences in signaling pathways and neurotrophic factor levels in the brains of HB-EGF knockout mice. Although limited to rodents, these pre-clinical investigations suggest that HB-EGF might play a role in normal human brain development, function, and/or response to injury. Toxicology studies performed in cynomolgus monkeys did not predict human neuropsychiatric AEs, despite use of doses at up to two orders of magnitude higher than those used in the current clinical study [data on file, Kyowa Hakko Kirin, Inc.]. Furthermore, it was not possible to identify any KHK2866-specific binding in cross reactivity studies using cynomolgus monkey and human tissues as HB-EGF was extensively expressed, which may have masked any potential off-target binding which may be associated with the neurotoxicity.

Following conclusion of the clinical trial, two additional monkey studies were conducted to elucidate the pathophysiology of the neurotoxicity (unpublished data on file, Kyowa Hakko Kirin, Inc.). KHK2866 shows high binding affinity to primate (human and cynomolgus monkey) HB-EGF and none to rodent (mouse and rat) HB-EGF as the amino acid sequence of HB-EGF differs between primates and rodents. Neurotoxicity studies could not therefore be conducted in rodents. In the first study in cynomolgus monkeys, distribution of KHK2866 into the brain and cerebrospinal fluid was measured with ^{89}Zr-labelled-KHK2866 using immuno-positron emission tomography. This revealed that there was no significant distribution to the brain; distribution was mainly to the heart, liver, kidney and spleen. In the second study, KHK2866 (2 mg total) was infused directly into the brain of normal cynomolgus monkeys by intracerebroventricular dosing. There were mild behavioral symptoms in some animals,

which were weakly similar to the symptoms seen in the clinical study. At this time, the mechanism of the observed neurotoxicity in the clinical trial has not been identified.

We have been unable to identify any other therapeutic MAbs in the literature that induce a similar spectrum of neuropsychiatric toxicity (apart from isolated or infrequent effects) as that experienced with KHK2866. It is likely, however, that any therapeutic product inducing such toxicity during early phase trials would have precluded further clinical development and publication of results. U3-1565, another fully human anti-HB-EGF MAb, has been investigated in a phase 1 study in patients with advanced solid tumors: no DLTs were observed, MTD was not reached, and no neuropsychiatric AEs were mentioned in the report [42]. The difference between KHK2866 and U3-1565 with respect to the induction of neurotoxicity is not known given they are both humanized anti-HB-EGF MAbs. It is known that KHK2866 binds to both soluble and membrane-bound forms of HB-EGF [32, 33], while the target(s) of U3-1565 is unknown. It is also not known if the two MAbs bind to similar epitopes.

Treatment with KHK2866 revealed durable stable disease in some patients, including three patients with confirmed stable disease. During an interim analysis of 20 patients, three completed at least six cycles of therapy and two of these had stable radiographic tumor imaging for a full 6 months, both of whom had squamous cell carcinomas. The protocol was therefore amended to include patients with squamous cell carcinomas, as well as those with EOC, in the MTD expansion cohort and reduced dose cohort (0.1 mg/kg), although the study was terminated shortly thereafter.

In conclusion, this study was terminated because of the occurrence of a syndrome of neuropsychiatric AEs associated with weekly dosing of KHK2866. The only factor associated with neuropsychiatric toxicity was KHK2866 administration itself. This effect was not dose-dependent. Although all patients experiencing these particular AEs recovered with time, these events were not predictable, their etiology is not presently understood, and there is no known treatment other than the passage of time.

Acknowledgments Medical writing assistance provided by Peter Todd of Tajut Ltd. (Kaiapoi, New Zealand) was supported financially by Kyowa Hakko Kirin Inc. during the preparation of this manuscript. The Institute for Drug Development, Cancer Therapy, and Research Center at University of Texas Health Science Center San Antonio (San Antonio, TX, USA) received support from Cancer Center Support Grant P30CA054174.

Compliance with Ethical Standards

Funding Kyowa Hakko Kirin, Inc.

Conflict of Interest XZ, HK, and VS are employees of Kyowa Hakko Kirin. PB was an employee of Kyowa Hakko Kirin until 29 August 2014. JS, MMM, MJB, LDC, LTC, and LHC declare that they have no conflict of interest

References

1. Pegram MD, Konecny G, Slamon DJ (2000) The molecular and cellular biology of HER2/neu gene amplification/over-expression and the clinical development of herceptin (trastuzumab) therapy for breast cancer. Cancer Treat Res 103:57–75
2. Mendelsohn J, Baselga J (2006) Epidermal growth factor receptor targeting in cancer. Sem Oncol 33:369–85
3. Slamon DJ, Leyland-Jones I, Shak S et al (2001) Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that over-expressed HER2. N Engl J Med 344:783–92
4. Fukuoka M, Yano S, Giaccone G et al (2003) Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non-small-cell lung cancer (The IDEAL 1 Trial). J Clin Oncol 21:2237–46
5. Cunningham D, Humblet Y, Siena S et al (2004) Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. N Engl J Med 351:337–45
6. Shepherd F, Pereira JR, Ciuleanu T, National Cancer Institute of Canada Clinical Trials Group et al (2005) Erlotinib in previously treated non-small cell lung cancer. N Engl J Med 353:123–32
7. Geyer CE, Forster J, Lindquist D et al (2006) Lapatinib plus capecitabine for HER-2 positive advanced breast cancer. N Engl J Med 355:2733–43
8. Van Cutsem E, Peeters M, Siena S et al (2007) Open-label phase III trial of panitumumab plus best supportive care compared with best supportive care alone in patients with chemotherapy-refractory metastatic colorectal cancer. J Clin Oncol 25:1658–64
9. Higashiyama S, Iwabuki H, Morimoto C, Hieda M, Inoue H, Matsushita N (2008) Membrane-anchored growth factors, the epidermal growth factor family: beyond receptor ligands. Cancer Sci 99:214–20
10. Yotsumoto F, Yagi H, Suzuki SO et al (2008) Validation of HB-EGF and amphiregulin as targets for human cancer therapy. Biochem Biophys Res Comm 365:555–61
11. Hurwitz H, Fehrenbacher L, Novotny W et al (2004) Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med 350:2335–42
12. Sandler A, Gray R, Perry MC et al (2006) Paclitaxel-carboplatin alone or with bevacizumab for non-small cell lung cancer. N Engl J Med 355:2542–50
13. Rini BI, Halabi S, Rosenberg JE et al (2008) Bevacizumab plus interferon alfa compared with interferon alfa monotherapy in patients with metastatic renal cell carcinoma: CALGB 90206. J Clin Oncol 26:5422–8
14. Friedman HS, Prados MD, Wen PY et al (2009) Bevacizumab alone and in combination with irinotecan in recurrent glioblastoma. J Clin Oncol 27:4733–40
15. Bublil EM, Yarden Y (2007) The EGF receptor family: spearheading a merger of signaling and therapeutics. Curr Opin Cell Biol 19:124–34
16. Goishi K, Higashiyama S, Klagsbrun M et al (1995) Phorbol ester induces the rapid processing of cell surface heparin-binding EGF-like growth factor: conversion from juxtacrine to paracrine growth factor activity. Mol Biol Cell 6:967–80
17. Miyamoto S, Yagi H, Yotsumoto F, Kawarabayashi T, Mekada E (2006) Heparin-binding epidermal growth factor-like growth factor as a novel targeting molecule for cancer therapy. Cancer Sci 97: 341–7
18. Sato M, Narita T, Kawakami-Kimura N et al (1996) Increased expression of integrins by heparin-binding EGF like growth factor in human esophageal cancer cells. Cancer Lett 102:183–91
19. Mishima K, Higashiyama S, Asai A et al (1998) Heparin-binding epidermal growth factor-like growth factor stimulates mitogenic

signaling and is highly expressed in human malignant gliomas. Acta Neuropathol 96:322–8
20. Ito Y, Higashiyama S, Takeda T, Yamamoto Y, Wakasa KI, Matsuura N (2001) Expression of heparin-binding epidermal growth factor-like growth factor in pancreatic adenocarcinoma. Int J Gastrointest Cancer 29:47–51
21. Ito Y, Takeda T, Higashiyama S, Noguchi S, Matsuura N (2001) Expression of heparin-binding epidermal growth factor-like growth factor in breast carcinoma. Breast Cancer Res Treat 67:81–5
22. Ito Y, Takeda T, Higashiyama S et al (2001) Expression of heparin binding epidermal growth factor-like growth factor in hepatocellular carcinoma: an immunohistochemical study. Oncol Rep 8:903–7
23. Ongusaha PP, Kwak JC, Zwible AJ et al (2004) HB-EGF is a potent inducer of tumor growth and angiogenesis. Cancer Res 64:5283–90
24. Itoh Y, Joh T, Tanida S et al (2005) IL-8 promotes cell proliferation and migration through metalloproteinase cleavage proHB-EGF in human colon carcinoma cells. Cytokine 29:275–82
25. Yagi H, Yatsumoto F, Miyamoto S (2008) Heparin-binding epidermal growth factor-like growth factor promotes transcoelomic metastasis in ovarian cancer through epithelial-mesenchymal transition. Mol Cancer Ther 7:3441–51
26. Tanaka Y, Miyamoto S, Suzuki SO et al (2005) Clinical significance of heparin-binding epidermal growth-like factor and a disintegrin and metalloprotease 17 expression in human ovarian cancer. Clin Cancer Res 11:4783–92
27. Suganuma K, Kubota T, Saikawa Y et al (2003) Possible chemoresistance-related genes for gastric cancer detected by cDNA microarray. Cancer Sci 94:355–9
28. Wang F, Liu R, Lee SW, Sloss CM, Couget J, Cusack JC (2007) Heparin-binding EGF-like growth factor is an early response gene to chemotherapy and contributes to chemotherapy resistance. Oncogene 26:2006–16
29. Yagi H, Yotsumoto F, Sonodo K, Kuroki M, Mekada E, Miyamoto S (2009) Synergistic anti-tumor effect of paclitaxel with CRM197, an inhibitor of HB EGF, in ovarian cancer. Int J Cancer 124:1429–39
30. Sloss CM, Wang F, Palladino MA, Cusack JC Jr (2010) Activation of EGFR by proteasome inhibition requires HB-EGF in pancreatic cancer cells. Oncogene 29:3146–52
31. Shinkawa T, Nakamura K, Yamane N et al (2003) The absence of fucose but not the presence of galactose or bisecting N-acetylglucosamine of human IgG1 complex-type oligosaccharides shows the critical role of enhancing antibody-dependent cellular cytotoxicity. J Biol Chem 278:3466–73
32. Miyamoto S, Iwamoto R, Furuya A et al (2011) A novel anti-human HB-EGF monoclonal antibody with multiple antitumor mechanisms against ovarian cancer cells. Clin Cancer Res 17:6733–41
33. Kasai N, Yoshikawa Y, Enokizono J (2014) Effect of antigen-dependent clearance on pharmacokinetics of anti-heparin-binding EGF-like growth factor (HB-EGF) monoclonal antibody. MAbs 6:1220–8
34. Eisenhauer EA, Therasse P, Bogaerts J et al (2009) New response evaluation criteria in solid tumors: revised RECIST guideline (version 1.1). Eur J Cancer 45:228–47
35. Rustin GJ, Quinn M, Thigpen T et al (2004) Re: new guidelines to evaluate the response to treatment in solid tumors (ovarian cancer). J Natl Cancer Inst 96:487–8
36. Kasai N, Kobayashi K, Shioya S et al (2012) Soluble heparin-binding EGF-like growth factor (HB-EGF) detected by newly developed immuno-PCR method is a clear-cut serological biomarker for ovarian cancer. Am J Transl Res 4:415–21
37. Mishima K, Higashiyama S, Nagashima Y et al (1996) Regional distribution of heparin-binding epidermal growth factor-like growth factor mRNA and protein in adult rat forebrain. Neurosci Lett 213:153–6
38. Nakagawa T, Sasahara M, Hayase Y et al (1998) Neuronal and glial expression of heparin-binding EGF-like growth factor in central nervous system of prenatal and early postnatal rat. Brain Res Dev Brain Res 108:263–72
39. Cantarella C, Cayre M, Magalon K, Durbec P (2008) Intranasal HB-EGF administration favors adult SVZ cell mobilization to demyelinated lesions in mouse corpus callosum. Dev Neurobiol 68:223–36
40. Oyagi A, Oida Y, Kakefuda K et al (2009) Generation and characterization of conditional heparin-binding EGF-like growth factor knockout mice. PLoS One 4, e7461
41. Oyagi A, Moriguchi S, Nitta A et al (2011) Heparin-binding EGF-like growth factor is required for synaptic plasticity and memory formation. Brain Res 1419:97–104
42. Moore KN, Bendell JC, Olszanski AJ et al. Phase I, first-in-human, dose-escalation study of U3-1565, a fully human anti-HB-EGF monoclonal antibody, in patients with advanced solid tumors. J Clin Oncol. 2013; 31 (suppl; abstr 2519).